THE EFFECTS OF EGG-WHITE AND ITS SPLIT PRODUCTS ON ANIMALS; A STUDY OF SUSCEPTIBILITY AND IMMUNITY.*†

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As is shown by the bibliography appended to this paper much of the research work done in the hygienic laboratory of the University of Michigan for some years past has dealt with the detection and extraction of a poisonous group in the proteid molecule. At first we worked with bacterial proteids and later we extended our research to animal and vegetable proteids. In each and all of these we have found poisonous bodies which we have extracted by various methods and obtained in differing degrees of purity. The purpose of this contribution is to report what we have done in the way of splitting up egg-white, to note the effects of the split products on animals, and to correlate the facts observed with those ascertained in our studies of other proteids.

Preparation of the egg-white.—Twenty dozen fresh eggs were broken and the whites dropped into 96 per cent alcohol. The coagulum was frequently stirred and repeatedly extracted with fresh quantities of alcohol. The coagulum was then air-dried on layers of filter paper, pulverized, placed in large Soxhlets, and thoroughly extracted with ether, after which it was again dried, pulverized, and kept in stock in large wide-mouth bottles.

Cleavage of egg-white.—The material prepared after the manner given above is placed in large flasks fitted with reflux condensers and extracted three times with from 15 to 25 times its weight of absolute alcohol in which 2 per cent of sodium hydroxid has been dissolved. These extractions are made at 78° C., the temperature of boiling absolute alcohol. This splits the egg-white into poisonous and non-poisonous portions, the former being soluble in the alcohol, while the latter remains insoluble in this menstruum.

THE POISONOUS PORTION

The alcoholic solution, after separation by filtration from the non-poisonous portion, is carefully neutralized with hydrochloric acid which converts the sodium into a chlorid. The precipitated chlorid having been removed by filtration, the filtrate is evaporated either in vacuo or on the open water-bath. The poison remains as a brownish deposit, containing more or less sodium chlorid, from which it

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may be further purified by resolution in absolute alcohol and re-evaporation.

The poison is soluble in absolute alcohol and in water, more readily in the former than in the latter. Its alcoholic solution is perfectly clear, but on standing forms a brownish deposit, which, however, is non-poisonous. An aqueous solution is opalescent, but becomes clear on filtration. The poison, both in powder and in solution, has a peculiar, penetrating, disagreeable odor, which seems to be the same in all the poisons that we have obtained from bacterial, animal, and vegetable proteids. The powder is brownish and deliquescent. Aqueous solutions are decidedly acid to litmus and slowly decompose sodium bicarbonate.

Aqueous solutions of the poison give all the color reactions for proteids except that of Molisch, and the carbohydrate group of the proteid molecule evidently remains in the non-poisonous group in the cleavage process to which we have subjected it. Whether or not the poison is to be regarded as a proteid is a matter that might be discussed. The fact that it is freely soluble in absolute alcohol has a negative bearing which is offset by its response to the color reactions. Its aqueous solutions are precipitated by uranyl acetate and metaphosphoric acid, and when these precipitations are complete the filtrates are not poisonous. The uranyl precipitate is somewhat soluble in alkaline carbonates. We think that the poisonous substance should at present at least be regarded as a proteid. When the poison is heated for some hours with equal parts of alcohol and hydrochloric acid it is destroyed, or, at least, in part robbed of its poisonous properties. From its alcoholic solution the poison is precipitated unchanged by ether.

The poisonous portion of egg-white is purified according to the methods detailed in earlier papers on the subject, and the intensity of its action does not differ, widely at least, from that of the poisons obtained from other proteids. Moreover, the symptoms induced in animals are the same and follow in the same order as when the poisonous portions of other proteids are used. The poisonous groups in all proteids are probably not identical chemically, but consist of closely allied bodies. The poison of egg-white kills just as promptly as that of the colon or typhoid bacillus. There are the same three

stages in the development and progress of the symptoms. At first there are evidences of peripheral irritation, indicated by the unaccustomed and sometimes violent scratching in which the animal indulges. In the second stage there is more or less marked paralysis of the muscles of the trunk and extremities, more noticeable as a rule in the posterior extremities. During this period the animal lies on its abdomen or side and when it moves, it may have some difficulty in co-ordinating its muscular contractions. Finally violent clonic convulsions supervene and death from failure of respiration occurs within from five to sixty minutes after the administration of the poison.

Animals may recover after passing through the first and second stages and after having been apparently very near death; but it is exceedingly rare to have an animal recover after the convulsions have begun. Although the minimum fatal dose is small, ranging from 8 or 10 to 100 mg. given intra-abdominally according to the degree of purification reached, the range between the smallest dose necessary to induce symptoms and that necessary to kill is wide. With one preparation the fatal dose was 70 mg., but 5 mg. developed the first and second stages in well-defined forms. It will be well to bear this in mind for its bearing upon some points that will be developed later in this paper. Like the other proteid poisons, that of egg-white has no apparent effect when administered by the mouth, or when inclosed in collodion sacs, it is introduced into the abdominal cavity.

When the proteid poisons are administered subcutaneously or intra-abdominally and not in large enough doses to cause too speedy death there is a marked fall in the temperature of the animal, but when the dose is overwhelmingly large, or when the poison is administered intravenously, the fall in temperature does not occur. This was noticed by V. C. Vaughan, Jr.,[17] in his study of the action of the intracellular poison of the colon bacillus, and is explained by the suddenness with which the fatal symptoms result. The fall in temperature does not occur, or occurs only rarely, when an animal is killed by a second injection of egg-white, because in this case the poison is liberated within the blood-vessels, and the process is equivalent to an intravenous injection of the free poison. When death is unusually delayed, as it is in some refractory animals sensitized to egg-white, the temperature does fall.

Chronic poisoning with this portion of egg-white or with like portions of other proteids is quite as interesting as the acute form, but as yet we have not given it the attention it deserves. Animals to which repeated non-fatal doses have been given lose flesh, become soft and flabby, are subject to skin eruptions, and die apparently from marasmus. We have seen these effects in guinea-pigs, rabbits, and goats which we have attempted to immunize to these poisons.

THE NON-POISONOUS PORTION.

The part of the egg-white left insoluble in the alcohol is purified after methods given in previous papers, and may be kept either in powder or in solution quite indefinitely without appreciable loss of its characteristic properties. It is largely, not altogether, soluble in water, and its aqueous solutions respond to all the proteid color reactions. The Molisch test, indicating the presence of the carbohydrate group, is given promptly and clearly. So far we have not made any determination of the percentage of carbohydrate in the egg-white residue, but it contains all that originally and naturally exists in the unbroken egg-white. An aqueous solution of this residue is precipitated by strong alcohol, and in this way the residue may be divided into two portions, one soluble and the other insoluble in dilute alcohol. This, as we shall see later, is not a quantitative separation, and each portion is probably a mixture.

INJECTIONS OF EGG-WHITE INTRAPERITONEALLY IN ANIMALS.

A single injection of egg-white into the peritoneal cavity of a rabbit or guinea-pig has no visible effect on the animal. This is true even when the volume is relatively large. Uhlenhuth,* in his studies on specific precipitins, injected at one time into the peritoneal cavity of rabbits the whites of from two to three eggs, diluted to 100 c.c. with physiological salt solution, and found that this treatment was well borne. Indeed, he repeated these injections at intervals of a few days and obtained from the animals thus treated a serum which gave a precipitum when added to egg-albumen diluted with 100,000 volumes of water. This experiment, though in a less heroic way, has been duplicated in many laboratories. We have repeatedly injected into the

* *Deutsch. med. Wchnschr.*, 1900, 26, p. 734.

abdominal cavities of half-grown guinea-pigs 10 c.c. of a dilution of egg-white with an equal volume of either sterilized water or physiological salt solution. We have not had occasion to use larger injections, and it has always seemed that 10 c.c. of fluid is as much as should be injected at one time into the abdominal cavity of a half-grown guinea-pig.

When the peritoneal injections are repeated at intervals of from two to five days for some weeks, the sera of animals thus treated give specific precipitums with solutions of egg-white, and this is regarded as evidence that the animal has acquired the function of digesting and assimilating or otherwise disposing of the foreign proteid introduced in this unusual way into the body. There is no reason for believing that this assumption is incorrect, but recent experiments have given additional interest to this subject.

A paper by Rosenau and Anderson* suggested to us that we might test our poisonous and non-poisonous portions of egg-white obtained as has been stated, by splitting up this substance with dilute alkali dissolved in alcohol.

Rosenau and Anderson found that if an interval of ten days or more elapsed between the first and second injection of serum the effect of the latter upon the animal would be serious and in many instances fatal. These authors say: "The first injection of horse serum has sensitized the animal in such a way as to render it very susceptible to a toxic principle in serum. It is probable that when the guinea-pig is injected with the first, or sensitizing quantity of serum the strange proteid contained in the horse serum develops in the body of the guinea-pig 'antibodies,' which, when brought into contact with more horse serum given at a second injection, produce either a union or a reaction, which cause the toxic action."

Several investigators have noted the peculiar behavior of animals under repeated injections of proteid material of diverse origin. Wolff† injected into rabbits both subcutaneously and intraperitoneally from 3 to 5 c.c. of emulsions of spleen, lymph glands, and bone marrow of calves, and noticed that the first injection was well borne by all, the second by most, but that all the animals died from the effects of the injections, from the third to the fifth, The symptoms observed were practically the same as those we have given as following the injection of our proteid poisons. Otto‡ reported the following findings: (1) With an initial dose of from 0.002 to 0.0025 c.c. of antitoxic serum and diphtheria toxin, an interval of from 4½ to 12 weeks, and a second dose of 6 c.c. of normal horse serum, 50 per cent of the animals died. (2) With a first dose varying from 0.235 to 6.4 c.c. of horse serum with diphtheria toxin, an interval of from six to 14 weeks, and a second dose of 6 c.c. of normal horse serum, all the animals died. (3) With a first injection of horse serum, an interval of from 5 to 10

* *Bull. Hygienic Laboratory*, 29, April, 1906.

† *Centralbl. f. Bakt.*, 1904, 37, p. 390.

‡ *Das Theobald Smithsche Phänomen, v. Leuthold Gedenkschrift.*

weeks, and a second injection of 6 c.c. of rabbit, goat, or ox serum, no reaction occurred in any of the animals. (4) With the first injection of a non-fatal dose of diphtheria toxin, an interval of from four to 11 weeks, of 34 guinea-pigs, 32 gave no reaction and two died. (5) With a first injection of toxone, an interval of from six to 10 weeks, and a second dose of horse serum, no reaction resulted. (6) With a first dose of horse serum varying from 0.0025 to 10 c.c., an interval of from 4½ to 14 weeks, and a second dose of from 6 to 7 c.c. of horse serum, it was found that none of the animals that had a large first dose reacted. Behring and Kitashima* reported the death of a horse during the progress of its immunization, notwithstanding the fact that at the time the serum of the animal was highy antitoxic. There can now be but little, if any, doubt that the horse was sensitized to the proteids contained in the culture medium. Brieger† reported that a goat died of tetanus after having been highly immunized to the toxin of that disease. It would certainly be very easy to mistake the convulsions of an animal dying from a subsequent dose for the spasms of tetanus. Rist‡ says that a guinea-pig bears without apparent injury 0.01 c gm. of the dried diphtheria bacilli, but 0.05 c.gm. cause progressive loss from which the animal begins to recover in about one week. But if after apparently complete recovery the same dose be given the effect is more marked; paralysis may develop and a third injection of the same amount given a month after the second causes death within from 24 to 48 hours. One pig received intraperitoneally without visible harm 0.01 c.gm. of the dried bacilli; on the 11th day after this it received 0.02 c.gm., which caused loss of weight, but the animal returned to the normal by the 32nd day. On the 36th day the animal received 0.05 c.gm., which led to its death within 24 hours. Arthus§ reported the untoward effects of repeated injections of horse serum in rabbits. He found that a single injection, large or small, caused no visible injury, and the conclusion is that horse serum is not toxic to rabbits. But he also found that a second injection given some days after the first did cause symptoms, light or grave, local or general, immediate or remote, according to the method of giving the second dose, subcutaneously, intra-abdominally, or intravenously, and he concluded that horse serum is toxic to a rabbit sensitized (*anaphylactisé*) to horse serum. The word, "anapylaxie" was first used by Richet and Portier to designate the condition of hypersusceptibility engendered in dogs to the poison of the tentacles of actinia by a previous treatment with the same poison. Later, Arthus and Breton¶ made an interesting report on the skin lesions induced in rabbits by repeated injections of horse serum. Around the point of the subcutaneous injection the tissue became infiltrated and in the more severe cases aseptic gangrene resulted. One of us, 10 years ago, in treating tuberculous individuals with yeast nucelic acid, had more than one fright from the untoward effects of an injection. Immediately after the injection the patient's face would flush and the erythema would rapidly extend over the entire surface of the body. This was accompanied by rapid, shallow breathing. In some instances an urticarial rash followed the erythema and lasted for some hours, but in the majority of instances the untoward symptoms disappeared almost as rapidly as they came and within a few minutes the patient was able to arise and walk home. At that time it was supposed that the effects were due to unintentional

**Berl. lin. Wchnschr.*, 1901, 38, p. 157.

† Quoted by Otto, p. 9.

‡ *Comp. Rend. de la soc. biol..*, 1903, 55, p. 978.

§ *Comp. Rend. de la soc. biol.*, 1903, 55, p. 817.

¶ *Comp. rend. de la soc. biol.*, 1903, 55, p. 1478.

intravenous injections, but the fact is now recalled that these symptoms were most frequently observed when several days elapsed between injections.

As we are writing this, Dr. Cumming, the assistant in this laboratory in charge of the Pasteur treatment of rabies, calls our attention to the following interesting case: E. M., of Dayton, O., aged 13, was treated in January, 1906, on account of the bite of a rabid dog. In March, 1907, the boy returned, having been bitten by a rabid cat. No unusual symptoms developed in the first treatment, but in the second treatment nothing was observed until the fourth day, when there developed about the point of injection an area of aseptic inflammation about three inches in diameter. The swelling became noticeable about six to seven hours after the injection and gradually disappeared after 48 hours. This resulted from every injection subsequent to the fourth. The treatment was continued notwithstanding these local effects and no harm seemed to be done. The sister of this boy had her first treatment at the same time that he was having the second, and received the same emulsions and in the same amount without any local or other reaction.

The effects of repeated injections of antidiphtherial serum, with intervals of from several days to years between treatments, have been reported tby Otto (*loc. cit.*), by Pirquet and Shick,* Rolleston,† and Currie.‡ The most elaborate and valuable of these reports are those of Pirquet and Shick. It should be stated that no fatal result has been reported due to repeated injections of antidiphtherial serum, but grave symptoms have been observed. Pirquet and Shick reported 61 cases with the interval between treatments of from 12 days to 7½ years. Under 12 days there is no reaction. In 30 cases in which the interval was between 12 and 50 days all showed an immediate reaction. In 11 cases with an interval of from two to six months there were both immediate and accelerated reactions and in 19 cases with an interval of from seven months to 7½ years there was an accelerated reaction. By an accelerated reaction is meant the development of symptoms which may follow a single injection, but which come on more promptly and are more severe.

Besredka and Steinhardt§ have made an interesting contribution to the literature of hypersusceptibility. They believe that the first injection of horse serum, although apparently harmless, produces a brain lesion, which renders this organ especially susceptible to the second injection, and they prove this, apparently to their own satisfaction, by showing that the second dose is more surely fatal when given subdurally, but they fail to explain why a period of from 10 to 12 days must elapse before this brain lesion becomes susceptible to the second dose. Furthermore, they have no difficulty in establishing what they call immunity or a condition of antisusceptibility. One of their experiments is detailed as follows: Two guinea-pigs received eight days after sensitization an intraperitoneal injection of 5 c.c. of horse serum without being affected. One received 10 days later and the other 14 days later subdurally 0.25 c.c. of serum and, as a result of this both animals, although visibly incommoded, soon recovered, while a control that had not received the second intraperitoneal injection—which they call a vaccination—died within five minutes. They might have observed the same phenomenon had the third dose been given intra-abdominally or subcutaneously. However, the contribution is a valuable one and shows two things: (1) the interval

* "Die Serum-krankheit 1905," *Münch. med. Wchnschr.*, 1906, 53, p. 67.

† *Practitioner*, 1905, 74, p. 664.

‡ *Jour. Hyg.*, 1907, 7, p. 35.

§ *Ann. de l'Inst. Past.*, 1907, 21, p. 117.

between the first and second dose must, in order to establish full sensitization, be as long when the second dose is given subdurally as when it is given subcutaneously or intra-abdominally. (2) Their results indicate that the poison that kills a sensitized animal does so by its action on the brain. We have repeatedly emphasized this second point in previous papers on the action of the poison obtained in a free state by splitting up the proteid molecule in the retort. Nicolle* has made a valuable report, but as it deals with repeated injections with short intervals it has but little bearing on the points that we are to discuss and we refer the reader to the original.

In our experiments with egg-white we have used material obtained directly from fresh eggs and the split products secured by the method already given. The fresh material has been drawn from newly laid eggs, diluted with an equal volume of either sterilized water or salt solution and filtered through paper. Our most important results may be formulated as follows:

1. One injection of egg-white is without apparent effect upon guinea-pigs, but sensitizes these animals after an interval of 10 to 12 days to a second injection. This is shown by the following:

TABLE 1.

All these animals received a sensitizing dose of 5 c.c. of the egg-white dilution and a second dose of the same amount, leaving the time interval between the injections the only controllable variable.

No.	Gms. Weight	No. Days Interval	Result
360	315	2	Not affected
361	335	4	" "
363	325	6	" "
368	320	8	" "
1	275	12	Died within 40 min.
5	355	27	" " 40 "
33	495	62	" " 30 "
28	305	92	" " 20 "
56	485	125	" " 20 "
52	310	154	" " 18 "

2. One c.c. of the egg-white dilution serves as a sensitizing dose, just as well as five times that amount.

It will be seen by comparing Tables 1 and 2 that, notwithstanding the difference in the sensitizing doses, the effects are the same. There is the same interval between the time of administration of the sensitizing dose and the development in the animal of the condition of sensitization, and when developed this seems to be the same both in intensity and in permanency.

3. However, with wider ranges in the size of the sensitizing doses differences in effect may be observed.

* *Ibid.*, p. 128.

TABLE 2.

These animals received a sensitizing dose of 1 c.c. and a second dose of 5 c.c. of the egg-white dilution (1:1).

No.	Gms. Weight	No. Days Interval	Result
85	305	6	Not affected
86	225	8	" "
87	235	10	1st and 2d stages
88	235	12	Dead in 15 minutes
4	355	19	" " 15 "
8	275	26	" " 15 "
89	385	37	" " 65 "
91	290	47	" " 15 "
92	285	63	" " 50 "
93	230	79	" " 40 "
94	280	93	" " 8 "
95	280	105	" " 6 "
96	295	124	" " 12 "
97	245	152	" " 20 "

In studying the sensitization of animals the following factors should be taken into consideration: (1) The amount of the sensitizing dose. (2) The interval in time between the first and second doses. (3) The amount of the second dose. (4) The individuality of the animal. In the following table we have arranged a series according to the size of the sensitizing dose. The animals were all of practically like age at the time of sensitization. The second dose was the same in all. The two controllable variables are the size of the sensitizing dose and the time interval. It should be stated that the weights given in this and in all our lists are those of the animals at the time they received the sensitizing doses, and the amount of egg-white given is that of a dilution with an equal volume of either sterile salt solution or water, filtered through paper.

TABLE 3.

No.	Gms. Weight	1st Dose in c.c.	No. Days Interval	2d Dose in in c.c.	Result
52	310	0.02	13	5	1st and 2d stages
51	285	0.05	13	5	1st " 2d "
50	240	0.01	13	5	1st " 2d "
49	285	0.20	13	5	1st " 2d "
88	235	1	12	5	Dead in 15 min.
4	355	1	19	5	" " 15 "
7	285	2	13	5	" " 30 "
16	300	2	33	5	" " 15 "
3	315	2	13	5	1st and 2d stages
2	305	3	13	5	1st " 2d "
6	305	3	13	5	Dead in 30 min.
11	260	4	31	5	" " 40 "
15	290	4	39	5	" " 15 "
1	275	5	12	5	" " 40 "
28	305	5	92	5	" " 20 "
10	350	6	33	5	" " 35 "
14	360	6	45	5	" " 17 "
9	370	10	18	5	1st and 2d stages
13	335	10	56	5	Dead in 20 min.
21	305	10	13	5	1st and 2d stages

We are well aware of the fact that before positive and final conclusions are drawn the list must be a much longer one, but the following conclusions may be formulated tentatively: (1) The very small sensitizing doses, under 1 c.c. of the dilution, are not so efficient as the larger ones. (2) One c.c. of the dilution is quite as efficient as a sensitizing dose as a larger one. (3) The time interval is an important factor. (4) When the largest sensitizing doses are used the time interval must be longer in order for the second dose to kill.

4. The non-poisonous portion of egg-white, designated as "the residue," sensitizes to unbroken egg-white.

TABLE 4.

These animals received varying amounts of the residue as sensitizing doses, but all received 5 c.c. of the egg-white dilution (1 : 1) for the second dose. They are arranged according to the time interval.

No.	Gms. Weight	Amt. o. Res. in mg.	No. Days Interval	Result
99	295	25	2	Not affected
100	245	25	4	" "
101	240	25	6	" "
56	220	10	10	1st and 2d stages
55	260	25	10	Died in 12 min.
102	340	25	10	" " 18 "
54	295	50	10	Not affected
120	395	50	10	" "
53	285	100	10	Died in 15 min.
61*	260	50	11	Convulsions, but recovered
62	305	50	13	Died in 36 min.
103	290	25	15	" " 40 "
106	355	25	15	" " 15 "
121	420	50	15	" " 25 "
20	325	200	15	" " 20 "
19	340	300	15	" " 15 "
63	335	50	25	" " 17 "
64	315	50	25	" " 40 "
183	485	1	26	Not affected
185	570	1	26	Slightly affected
186	575	1	26	1st and 2d stages
184	605	1	26	Died in 23 min.
180	615	5	26	Not affected
179	495	5	26	Slightly affected
182	425	5	26	Died in 27 min.
175	515	10	26	" " 20 "
123	465	50	31	" " 15 "
124	655	50	41	Not affected
125	355	50	41	Died in 15 min.
126	660	50	41	Not affected
104	305	25	114	Died in 18 min.
105	315	25	114	" " 30 "
119	370	50	114	" " 15 "
132	390	50	114	" " 20 "

It will be noticed, in looking over this table, that there are a few animals, which even after an interval of 10 days or more are not sensitized. This occurs when unbroken egg-white is used for the sensitizing dose quite as frequently as it does when the non-poisonous

* No. 61 is the only animal that we have seen recover after convulsions appeared.

portion is employed for this purpose. We are of the opinion that the failure to sensitize with either the non-poisonous portion or the unbroken egg-white is due to the age of the animal, and that old animals are not so easily sensitized as the young. It will be observed on going over the table that all the animals that were not affected by the second dose after the proper time interval were old animals, as indicated by their weight. It is true that some old animals were sensitized, but it is equally true that all those that were not sensitized were old. We state this only as an opinion, and are fully aware of the fact that a much greater number of animals would be required to make a positive demonstration. If this opinion should prove to be a fact it will probably be found that the small sensitizing doses will prove much more efficient when young animals are used. It happened quite accidentally that the animals in which we used the smallest sensitizing doses were some which we secured because they were believed to be too old to breed and all were old males. At the time that we sensitized these animals we had not thought of age as a factor in sensitization, and it only occurred to us that this might be true after we had tabulated the results. This point is an interesting one and deserves further study.

5. The non-poisonous portion of the residue does not sensitize to itself as is shown by the following:

TABLE 5.

No.	Gms. Weight	1st Dose in mg.	No. Days Interval	2d Dose in mg.	Result
103.....	290	25	10	150	Not affected
104.....	305	25	15	250	" "
105.....	315	25	15	200	" "
119.....	370	50	10	250	" "
122.....	390	50	15	250	" "

No. 103 received five days after the second injection of the residue 5 c.c. of the egg-white dilution, from which it developed the characteristic symptoms and died in 40 minutes. This shows that the animal was sensitized by the first dose of the residue, because the sensitized condition requires more than five days for its development. Moreover, another animal (106) received 25 mg. of the residue at the same time, and 15 days later it had 5 c.c. of egg-white dilution from which

it died in 15 minutes. Nos. 104, 105, 119, and 122 received, 114 days after the sensitizing dose of the residue was given, 5 c.c. of egg-white dilution and died in 18, 30, 15, and 20 minutes, respectively. No. 103 suggests another interesting point, i. e., the second dose of the residue did not prevent the animal from splitting up unbroken egg-white given five days later, which would have been the effect had the second dose been a non-fatal one of egg-white.

The fact that the residue does not even in the slightest degree sensitize to itself, while it does fully sensitize to unbroken egg-white, demonstrates two things very clearly. First, it shows that our separation of the poisonous and non-poisonous portions is an actual and complete separation. If there remained in our non-poisonous portion any unbroken egg-white it should sensitize to itself, and the second dose, especially with the large quantities that we have used, should develop some symptoms even if it does not kill, for we have seen that one-fourteenth of the fatal dose of the poison develops to a marked degree the first and second stages. In the second place, it shows that only when the second dose contains the poisonous group does it develop symptoms. This, along with the fact that the symptoms induced by the split-off poison and those that follow the injection of unbroken egg-white into a sensitized animal are identical, proves conclusively, to us at least, that sensitization consists in rendering the animal capable of splitting up egg-white and that this cleavage as it occurs in the animal yields the same products that we obtain by our artificial method in the retort. Our method is undoubtedly crude compared with the cleavage process that occurs in the body of the sensitized animal, so far as quantity of active poison is concerned, for we are quite sure that by the artificial method we destroy more or less of the poison; but, so far as the quality of the poison yielded by the two processes goes, the results are identical.

6. There are reasons for suspecting that the sensitizer contains two bodies the joint presence of which is necessary for full sensitization.

We speak with caution on this point and recognize that additional work is necessary before we can make a positive statement. An aqueous solution of the residue was acidified with hydrochloric acid and treated with an equal volume of absolute alcohol. The pre-

cipitate produced by this treatment was collected and dried and the filtrate evaporated to dryness and these portions were employed separately and jointly in sensitizing animals with the following results.

TABLE 6.

No.	Gms. Weight	Amount in mg.	No. days Interval	No. c.c. of Egg-White	Result
PRECIPITATE.					
262	395		11	5	Not affected
263	370		25	5	1st and 2d stages
264	405		11	5	Slightly affected
FILTRATE.					
265	415	100	10	5	Slightly affected
266	405	50	24	5	1st and 2d stages
267	425	25	24	5	1st " 2d "
268	455	12.5	10	5	Slightly affected
COMBINATION.					
318	350	25 each	12	5	Died in 5 min.
319	360	25 "	12	5	" " 45 "

We have obtained similar results in immunizing animals to the colon bacillus with the non-poisonous part of the cellular substance of that bacterium; but, as we have stated, this point deserves further investigation.

7. The active substance in the sensitizer or residue is probably a proteid.

The evidence upon which this statement is founded was obtained from experiments in which all proteid was removed from aqueous solutions of the residue after which it failed to sensitize. After complete precipitation of an aqueous solution of the residue with uranyl acetate and removal of uranium from the filtrate with sodium phosphate, the portion of the residue left failed to sensitize animals. We have inserted the word "probably" in our statement, because we recognize that there remains the possibility that the sensitization may be due to some non-proteid group which is precipitated with the proteid by uranyl acetate. But we have found that whenever we remove from our residue the group or groups that give the proteid color reactions, it fails to sensitize.

8. The poisonous portion of the split products of egg-white either in single or in multiple doses, does not sensitize to unbroken egg-white.

TABLE 7.

These animals had 5 c.c. of the egg-white dilution after previous treatment with the poison.

No.	Gms. Weight	Amt. Poison in mg.	No. Days Interval	Result
41	490	12.5	36	Not affected
45	265	5	37	" "
33	495	25	11	" "

Other animals had multiple injections of the poison and after varying intervals from the time of the last injection received 5 c.c. of the egg-white dilution without effect. This demonstrates that no portion of the sensitizing group remains in the poisonous portion obtained by our method of separation. This test is more delicate than a chemical one, for had one mg. of the residue remained in the poisonous portion it would have sensitized the animals to unbroken egg-white, and some of our pigs that received multiple injections of poison were given altogether as much as 275 mg. of the poisonous portion. We, therefore, conclude that these experiments demonstrate two things: (1) The poisonous portion does not sensitize to unbroken egg-white, and (2) the poisonous portion as we have used it contains no trace of the sensitizing substance.

9. The second dose in order to kill must contain enough egg-white to furnish a fatal dose when split up in the animal body.

Inasmuch as the cleavage process in the animal body occurs in part at least in the blood, the amount of poison necessary to kill under these conditions is small—probably less than 5 mg., possibly less than half this amount—because it has been shown in our work on the colon poison that 10 mg. of the crude poison may kill within four min., when given intravenously, and we have learned that the pure poison does not constitute more than 15 per cent of the crude product. This would reduce the fatal dose when given intravenously to 1.5 mg. The minimum fatal dose cannot be greater than this and it may be smaller. It follows from this that the second dose may be small and yet cause a fatal result, but that it cannot be infinitesimally small and cause death. Indeed, the second dose of egg-white, in order to kill, must be an easily measurable quantity. There is no reason, so far as we can see, for holding or supposing that the second dose must act like a ferment, because it may be very small and yet kill. Whether or not the poison kills depends not only on the amount injected into

the animal body but the rapidity with which it is introduced. We have seen that this is so important a factor that when a quantity of the poison equivalent to several times a fatal dose measured by the effects when injected intravenously, intra-abdominally, or subcutaneously, is introduced into the stomach or sacked in the peritoneal cavity, it has no visible effect on the animal although the poison slowly diffuses unchanged through a collodion sac. Although we regard the effect of an intra-abdominal injection of egg-white into a sensitized guinea-pig as comparable in some respects to an intravenous injection of the free poison, we recognize that there are differences, and we would not expect an amount of egg-white which would furnish not more than 1.5 mg. of the free poison to kill a sensitized animal. In other words, if the minimum fatal dose of the poison when given intravenously be 1.5 mg., we would expect that the minimum fatal dose of egg-white in a sensitized animal would be an amount which on being split up would yield something more than this amount of the poison.

TABLE 8.

No.	Gms. Weight	1st Dose in c.c.	No. Days Interval	2d Dose in c.c.	Result
179.....	495	5	17	1	Slightly affected
180.....	615	5	17	1	" "
183.....	485	5	17	1	1st and 2d stages
185.....	570	5	17	1	1st " 2d "
380.....	470	5	18	1	1st " 2d "
381.....	485	5	18	1	1st " 2d "
382.....	475	5	18	1	1st " 2d "
383.....	465	5	18	1	1st " 2d "
384.....	445	5	18	1	1st " 2d "
385.....	440	5	18	1	1st " 2d "
386.....	500	5	18	1	1st " 2d "
387.....	490	5	18	1	1st " 2d "
388.....	515	5	18	1	1st " 2d "
389.....	515	5	18	1	1st " 2d "

Although egg-white when injected intra-abdominally in a sensitized animal acts speedily, its average action cannot be so rapid as that of the free poison injected directly into the blood. We have found, as the next table shows, that 1 c.c. of the egg-white dilution (1:1) does not kill adult sensitized pigs. Now 1 c.c. of this dilution contains not more than 500 mg. of egg-white and egg-white contains about 12 per cent of proteid. This egg-white does not contain so much proteid because it has been filtered through paper and a considerable proportion of the proteid has not passed through the paper.

But supposing that it did contain 12 per cent of proteid, then our injection contained 60 mg. of proteid, and we have shown that egg-white yields about one-third its weight of crude poison, and this would make 20 mg., and 15 per cent of this would be 3 mg., which must represent the maximum of pure poison that the 1 c.c. of the dilution could contain—it actually did not contain so much. It should be remarked that all proteids do not yield like amounts of the poisonous groups.

In comparing this list with others it must be evident that the size of the second dose has an important influence on the result, and that 1 c.c. of our egg-white dilution when split up in the body of an adult pig does not supply a fatal dose of the poison.

10. Animals that are sensitized and yet recover from the second injection of egg-white are affected by and may die from a third or a subsequent injection, provided the interval between treatments is long enough.

The animals in the following table illustrate this statement. The sensitizing doses ran from 2 to 10 c.c. of the egg-white dilution but all the subsequent doses were of the same size, except the last one given No. 3. It appears from this table that there is a minimum interval which must supervene between any two injections before the animal regains the sensitized state. Whether or not this interval is longer than that which is necessary between the first and second doses we have not sufficient experimental data for determining, but the indications are that the interval must increase with the number of treatments. With No. 2, the interval between the second and third doses was six days, and this was not enough for the animal to regain its condition of sensitization; but in all other instances in this list the animal was in a sensitized state when the dose was given it. A pig need not die in order to show that it is sensitized; the development of the first and second stages is sufficiently characteristic to demonstrate sensitization. The question why the ordinarily fatal dose does not kill the sensitized animal is apart from that whether or not the animal is sensitized. The sixth dose of egg-white made No. 2 quite as sick as the second one, and on the receipt of each the animal was in a sensitized state; indeed, the animal finally died from the sixth dose. Now, why was it that 5 c.c. of the egg-white dilution

did not kill any of these animals the first time it was given them after they had been sensitized? If this question can be answered satisfactorily it will also tell us why it failed to kill at any of the subsequent injections. The most ready answer to the question is that the 5 c.c. of the egg-white dilution failed to kill these pigs because it did not contain enough poison. The dose contained the amount of poison that usually kills sensitized pigs, but these are unusually resistant and do not die. Increase the dose of the egg-white and the animals will die. We did increase the dose in No. 3, and yet the animal did not die. The second dose must furnish a fatal amount of the poison when the proteid is broken up in order to kill, but the proteid may contain several times the fatal dose and still not kill the sensitized animal. In our opinion this is due to the rate at which the proteid is broken up and the poison set free. Animals that prove refractory after sensitization are those in which the proteolytic process for some reason proceeds slowly. We have observed that these refractory animals develop even the first stage of poisoning much later than it usually appears, and the second stage lingers a long time and these animals may die without passing through the convulsive stage. We have in these animals what we may designate as a subacute form of poisoning, and they die no quicker from 10 c.c. of the egg-white dilution than they do from half that amount. Indeed, we are not sure that the failure to kill No. 3 was not due to the fact that we doubled the last dose, but this brings up a question which we are not at present prepared to discuss and concerning which we hope to be able to speak more confidently soon.

11. The young born to sensitized parents inherit the condition of sensitization. We reserve the discussion of this point for a future paper.

Attempts were made to ascertain whether or not the ferment supposed to break up this egg-white could be detected in the body of a pig just dead from the effects of a second dose. These experiments while not wholly satisfactory are worthy of record and deserve repetition and extension.

The sterile salt washing from the peritoneal cavity of a pig just dead from a second dose was added to 10 c.c. of the egg white dilution, and the mixture after standing for 16 hours at 37° was injected

into the abdominal cavity of a fresh pig. The animal developed the first and second stages of poisoning but recovered.

TABLE 9.

No.	Gms. Weight	1st Dose in c.c.	No. Days Interval	Subsequent Dose in c.c.	Result
2.....	305	3	13	5	1st and 2d stages
			6	5	No effect
			21	5	1st and 2d stages
			30	5	1st " 2d "
			96	5	Died in 2½ hours*
3.....	315	2	13	5	1st and 2d stages
			16	5	1st " 2d "
			41	5	1st " 2d "
			96	10	1st " 2d "
5.....	355	5	13	5	1st " 2d "
			27	5	Died in 40 min.
9.....	370	10	18	5	1st and 2d stages
			27	5	Died in 45 min.
21.....	305	10	13	5	1st and 2d stages
			44	5	1st " 2d "
			96	5	Died in 56 min.
23.....	310	5	13	5	1st and 2d stages
			44	5	Died in 30 min.
24.....	295	5	13	5	1st and 2d stages
			44	5	1st " 2d "
			96	5	Died in 35 min.
25.....	310	5	13	5	1st and 2d stages
			44	5	Died in 20 min.

* No. 2 died more slowly than any other animal that we have killed with egg-white.

Five c.c. of the egg-white dilution mixed with the same amount of peritoneal washing from a pig just dead from a second dose was immediately injected into the abdominal cavity of a fresh pig. This animal developed the first and second stages but recovered.

Five c.c. of the egg-white dilution was rubbed up with the spleen of a pig just dead from a second dose and the fluid portion was immediately injected into the abdominal cavity of a fresh pig: No effect. Like results followed similar treatments with the adrenals and portions of the omentum and liver. We washed out the peritoneal cavities of three pigs just dead from second doses of egg-white with sterile salt solution and immediately injected these washings into the abdominal cavities of three fresh pigs. These animals immediately became restless, scratched, and kept up a continuous crying for some minutes and speedily recovered. The object in this experiment was to demonstrate, if possible, the existence of the free poison in the abdominal cavity of the dead guinea-pig, but the evidence was not positive and still leaves us in doubt. However, we could not expect to find any more than a trace of the poison in the peritoneal cavity of an animal dead from its effects. It is worthy of note that the ani-

mals treated with these peritoneal washings were thereby sensitized to unbroken egg-white, as was demonstrated by subsequent treatment. The sensitization may have been due to either whole egg-white or to the non-poisonous portion.

CHRONIC POISONING WITH EGG-WHITE.

It is not our purpose to enter minutely in this paper into the interesting subject of chronic poisoning with foreign proteids, but some general statements should be made. We have treated guinea-pigs daily with intra-abdominally injections of egg-white dilution (1:1). One set of pigs received daily 5 c.c. and another 2 c.c. of the dilution. These animals were apparently but little affected. Most of them lost weight, but some did not and others fluctuated, losing for a few days and then gaining. Two died after the eighteenth, six after the nineteenth and one after the twenty-first injection. It should be stated that those having 2 c.c. of the egg-white dilution died quite as promptly as those having 5 c.c. The post-mortem finding was uniform in all and quite interesting. It consisted of a hemorrhagic, aseptic inflammation of the omentum. Besides the omentum and the mesentery all other organs, macroscopically at least, appeared normal. There was in all instances a bloody fluid in the peritoneal cavity, and this was sometimes cloudy with coagulated fibrin. The parietal peritoneum was normal. It should be stated that paralysis and convulsions precede death much as they do after acute poisoning, but continue through a much longer time. If the body be properly sectioned, cultures made from the peritoneal cavity, omentum, liver, spleen, and heart's blood remain sterile. The localization of the inflammation in the omentum and mesentery is striking and characteristic. Evidently the continued absorption of the foreign proteids lead to congestion and finally to hemorrhage.

An animal that has received twenty of the smaller doses gets altogether only 2½ grams of albumen, and this given in divided doses kills. It must be evident from this that the repeated injection of foreign proteids into animals cannot be regarded as altogether free from ill effect.

INTERPRETATION OF RESULTS.

A correct interpretation of the phenomena which have been recorded in the preceding pages will be of much value to both the physiologist

and the pathologist; and at the risk of falling into error this will be attempted, with the provision that further study and elucidation of the facts, may lead to a modification of their explanation. We have by chemical agents outside of the animal body broken up the complex proteid molecule into two groups, one of which contains a poisonous substance, while the other group contains a body which sensitizes animals to the unbroken proteid. We, probably better than others, are fully conscious of the fact that our artificial method of splitting up the proteid is crude compared with the process that takes place in the body of the sensitized animal. We called attention to this some years ago, in discussing this question in connection with our work on the cellular substance of the colon bacillus and its cleavage products. At that time we stated that by our artificial method much of the poisonous body must be destroyed, or at least rendered inert, while in the animal body this does not occur, at least not to so great an extent. We do not suppose that by our artificial method we have broken up the proteid molecule into two and only two parts, one of which is haptophor or possessed of sensitizing properties, and the other toxophor, or a chemically pure body possessed of poisonous properties. Indeed, we know that this is not true, and that one group contains other than the haptophor and that the other contains, in addition to the toxophor, substances that are inert. Our work shows conclusively that our non-poisonous residue contains besides the sensitizing bodies substances that have nothing to do with inducing in the animal the condition which for the want of a better term we call sensitization, and with equal certainty our work shows that our poisonous substance is a mixture and not a chemically pure body. As yet we remain quite ignorant of the chemical composition and constitution of both the sensitizer and the poison, and the solution of these questions is a task still before us. However, with what has been done chemically outside of the body and with what has been learned by animal experimentation, we think that we have some basis upon which a theory may be, at least tentatively, offered. Furthermore, the observation of facts, the prosecution of laboratory investigations, and the sacrifice of animal life in experimentation are of little value unless we attempt to correlate the facts, systematize the chemical investigations, and study the relationship between cause and effect in our animal experimentation.

We can see no escape from the conclusion that the active agent in our toxophor obtained by chemical means from egg-white, and the substance that kills the animal sensitized to egg-white, when a second dose of this is administered, is one and the same. Both are constituents of the egg-white, and are groups in the same complex molecule. However, if one wishes to contend that egg-white is not made up of complex molecules, as we hold, but is a physical mixture of substances among which our haptophor and toxophor exist, he still cannot avoid the conclusion stated in the preceding sentence any more than he can escape the conviction that the man who dies from morphin and the one who dies from opium die from the same poison. The symptoms induced by the toxophor split off by chemical agents and those observed in the animal sensitized to egg-white on the second administration of this substance are identical in every particular. They originate in the same time, proceed in like order, and terminate alike. The mode of death is the same and the post-mortem findings in both are identical. We must, therefore, conclude that the process of sensitizing an animal consists in developing in its body a substance which affects the egg-white, just as the alcoholic solution of alkali does in the retort, but much more promptly and efficiently.

It is equally certain that our artificially obtained haptophor contains the substance which develops in the body of the animal the capability of speedily and effectively splitting up the egg-white, or, if one prefers, extracting and liberating its poisonous constituent. This is shown by the fact that our haptophor sensitizes animals to the poisonous action of egg-white quite as well as egg-white itself does. The poison in egg-white therefore has nothing to do with the sensitization of the animal, and that there is no poison left in our artificially prepared haptophor is shown by the demonstration that it does not sensitize to itself. It seems that the demonstration of these points is complete and incontrovertible.

That the egg-white is split up in the body of the sensitized animal is shown by the experiments in which unsensitized animals were affected by the injection of the peritoneal washings from sensitized animals just dead from injections of egg-white. We will admit that the evidence on this point has not been so clear and striking as we might wish, but it could not be expected that large amounts of the

free poison would be found in the peritoneal cavity of an animal just dead from its effects, any more than we should hope to recover from the stomach of a man who had just died from the minimum fatal dose of morphin an equal amount of that poison. That either unbroken egg-white or its haptophor remains in the peritoneal cavity of an animal dead from the effects of egg-white is shown by the fact, repeatedly demonstrated, that the washing from this cavity sensitizes fresh animals to egg-white.

That the cleavage agent does not exist, at least in effective amount, in the liver, spleen, adrenals, or omentum of the sensitized animals is indicated by the results of experiments already detailed. That the cleavage agent does not exist, during the intervals between injections, in the blood of the sensitized animal we infer from the failure to detect any trace of it in the blood serum obtained from sensitized animals during these intervals.

There is no evidence that an antitoxin is produced by single or repeated injections of either the unbroken egg-white, its toxophor, or its haptophor constituents. It is true that some slight increase in the resistance of the animal to the toxophor may be induced by repeated injections of this body seems well established, but that this is due to the production of an antibody we see no good reasons for believing. In the first place, this increased tolerance is at best only slight, it is not specific, and the attempt to detect an antibody in the blood serum of sensitized animals has uniformly failed. A sensitized animal is no more and no less susceptible to the toxophor than a fresh animal. Indeed, we can see absolutely no ground for believing that the toxophor has any concern in inducing the condition of susceptibility which we call sensitization. Quite naturally it occurred to us that the haptophor might elaborate an antibody to its own toxophor, but by neither single nor repeated injections of the haptophor or the unbroken egg-white have we been able to secure any confirmation of this possibility.

It seems that we must conclude that the introduction of egg-white or its haptophor constituent into the blood of an animal, by either intra-abdominal or subcutaneous injection leads to the production of a new digestive or proteolytic secretion. It leads to the development of a new function on the part of certain body cells, and

this new function consists in the elaboration of a secretion which breaks up egg-white in the animal body, very much as we have broken it up with the alcoholic solution of alkali but much more quickly and efficiently. This new proteolytic secretion is formed and held in certain cells in the body until these are stimulated by the reappearance of egg-white in the circulatory blood. This secretion belongs to that class of bodies which have long been designated as ferments or enzymes and its action is specific. It splits up egg-white and no other proteid. It is called into existence by the introduction of egg-white or its haptophor into the circulation of the animal. The introduction of this foreign proteid into the body calls for some means for its disposal. The body has no agent by which this can be accomplished, and, as a consequence, certain cells are called upon to produce such an agent. These cells respond to this call and begin to elaborate the needed enzyme. Gradually and somewhat slowly these cells acquire this new function. By means of this new enzyme the foreign proteid is split up and some of the split products probably serve certain cells in the body as food material, while others constitute a menace to health and even to life. Our haptophor group contains the sensitizing body, that which calls into existence this new function and leads to the elaboration of the new proteolytic enzyme. Our toxophor group contains the substance that endangers the life of the animal. As our experiments have shown, danger to the life of the animal depends not so much upon the amount of the free toxophor introduced into the animal as upon the amount set free at one time. When we first introduce egg-white or other foreign proteid it is slowly broken up and no recognizable harm is done the animal. The same is true when frequent injections are made at short intervals of time. But when this new function of splitting up a foreign proteid is called into existence and time enough is given for the cells concerned in the development of this new enzyme to store this up within themselves, where it probably exists not as an active enzyme, but as a zymogen, then a second portion of this same foreign proteid is introduced, the specific zymogen becomes an active enzyme, and the foreign proteid is split up so rapidly that enough of the toxophor body is set free within a given time to affect seriously the animal and possibly destroy its life.

At first we inclined to the opinion that the interval of 10 or 12 days that must elapse between the administration of the first dose and the development of the condition of full sensitization was due to some temporary immunity induced by the toxophor; but when we found that in the same time and no sooner the same degree of susceptibility could be established by using as the sensitizing agent our haptophor, instead of the unbroken egg-white, we were compelled to abandon this theory. Certainly this would be necessary unless we could show that our haptophor produces by itself an antibody to our toxophor, and after we had demonstrated that this does not happen we were compelled to conclude that neither our toxophor nor an antibody to it has anything to do with the production of the condition of sensitization, not even any influence upon the time period necessary to develop it.

It can easily be understood of what great benefit it is to the animal body to break up a foreign proteid with fulminating rapidity as soon as it gains access to the tissues of the animal, provided that the foreign proteid is a living one. And the only foreign proteids that find their way into the blood of the living animal under natural conditions are living proteids. Unless the body be possessed of the power of quickly breaking up the living proteid, the invader multiplies, feeds upon the proteids of the animal body, converting them into foreign proteids, and finally destroys the host by virtue of the strength gained from its host.

THE CORRELATION OF THE WORK ON EGG-WHITE WITH PREVIOUS WORK ON OTHER PROTEIDS

We have by the same agents in the retort split proteids of diverse origin into poisonous and non-poisonous portions. Some of these proteids are bacterial, others vegetable, and still others are animal. We think it probable that all proteids are made up of poisonous and non-poisonous groups, and that these are combined in the same molecule, much as an acid and a base combine to form a salt. Indeed, we are inclined to hold that a proteid is a highly complex body containing an acid and a basic group, each of which is still complex and of such a character that for the present we must regard each as a proteid in itself. The acid part of the proteid molecule is an active poison when detached from its basic part and injected in this uncom-

bined state into the animal. It acts in the same way when it is separated from its base in the animal body. The acid group owes its poisonous action to the avidity with which it combines with the basic constituents of some of the cellular proteids of the animal body. From the symptoms that follow the injection of the free toxophor into the animal body or the injection of the unbroken proteid into the body of a sensitized animal we infer that the poisonous group endangers the life of the animal by its ready combination with the basic constituents of the proteids of the cells in the respiratory center. The poisonous group, whatever may be the source of the proteid from which it is obtained, induces in the animal the same train of symptoms and death is due to the same cause—to failure of respiration. The acid character of the poisonous group is indicated by the fact that it is best split off from the whole proteid by alkalis and this indication of the acid nature is confirmed by the fact, so well demonstrated in one of the earlier papers from this laboratory, that it combines with alkalis, and when thus combined its poisonous action is reduced.

In another paper we have stated our reason for believing that life is molecular or at least that metabolism, the one essential phenomenon of life, is intramolecular and that every proteid molecule has its chemical nucleus or center of chemical energy and that the poisonous group constitutes this chemical nucleus. The chemical nucleus of each proteid differs in some respects from that of every other proteid and yet all resemble each other in the readiness with which they combine with other bodies and construct whole proteids. In the free state they may be regarded as acid radicals with unsatisfied valences, and this accounts for their fulminating action as poisons when introduced into the animal body in the free state or when set free in the body of the sensitized animal. They act as poisons by tearing off from other proteids their secondary or basic constituents, probably not in whole, but only in part. This interrupts the function of the cell of which the injured proteid in an essential part, and if this function be one, the continued operation of which is essential to the life of the animal, this interruption endangers life and may cause death. The injured proteid in the animal cell is not destroyed; it is only crippled, and if not too suddenly injured it repairs its injury and the animal

lives, although death may have been imminent. In this way we explain the immense importance of the rapidity with which the poison enters the circulation or the rate at which it is set free in the sensitized animal.

Turning now to the non-poisonous or haptophor or sensitizing or immunizing substance, we have found that previous treatment of animals with this portion of the proteid of the colon or of the typhoid bacillus gives specific immunity to the living organism, and that previous treatment with this portion of the egg-white proteid renders the animal susceptible to the next dose of the same proteid, so that it kills. In one instance the life of the animal is saved; in the other it is jeopardized, and in the majority of instances lost. These phenomena seem to be antipodal and yet if we interpret them aright they are identical. The colon or the typhoid bacillus is only a specific, living proteid. It is a proteid in an active, metabolic state, capable of absorbing, assimilating, and multiplying. The egg-white is the product of life and with the potentiality of again becoming a living proteid. The bacillus is made up of labile molecules, while the molecules of the egg-white have passed into a more stable condition. The one is in an active, the other in a resting state; the one is actively engaged in trading in energy, the other is temporarily at least quiescent, and yet both are proteids, markedly similar in their chemical composition and yet characterized by a specific difference. Both are essentially proteids, made up of an acid or poisonous chemical nucleus and a basic or non-poisonous group. The former in its effect upon animals is the same, whether derived from the bacillus or the egg-white, and the latter in the one instance induces specific immunity and in the other specific susceptibility. But the immunity and the susceptibility each consists in developing in the animal body the capability of splitting up a specific proteid. If the living proteid be split up before it has had time to multiply sufficiently to furnish a fatal quantity of the toxophor the animal lives and we say that it has been immunized. If the stable proteid be introduced into the animal body it develops a specific proteolytic ferment, and if enough of it to supply a fatal dose be injected after this function has been edveloped, the animal dies. The first or sensitizing dose of egg-white injected into the animal is split up or digested just as surely as is the second

dose, but the process proceeds so slowly that we can see no effects, and we say that the egg-white is without effect upon guinea-pigs when injected subcutaneously or intra-abdominally. But subsequent injections show how erroneous this conclusion is. The first dose of egg-white has in truth affected the animal profoundly, so profoundly that the effect not only persists in the individual for months and possibly for years, but may be transmitted to the next generation. We do not say that the animal is sensitized, unless some immediate and striking effect follows our treatment; but it seems to us that this view is not altogether correct and that it needs some modification. As we have repeatedly stated the immediate effect, especially a fatal issue, depends first of all upon the rate at which the proteid is split up in the animal, and this depends first of all upon the amount of available proteolytic ferment or rather zymogen stored up in the animal body at the time of the injection. It is probably true that at the time of the first injection there is none of this specific ferment stored in the animal body. It is hardly believable that any cells have preformed in them, even in infinitesimally small quantities, all the specific proteolytic zymogens ready for any emergency. But the foreign proteid must be disposed of, and this is accomplished by the development of a specific ferment. This ferment is developed slowly and digests the proteid of the first dose so slowly that the well-being of the animal is not visibly affected. If now time enough be allowed for the accumulation of a large amount of this specific zymogen in the body-cells and then a second dose of the same proteid be injected, it is split up into its poisonous and non-poisonous constituents and the former induces the symptoms and may cause death. It seems to us that this satisfactorily explains most, if not all, of the phenomena observed in the study of the sensitization of animals to proteids. It has been noted by others as well as by ourselves that when the sensitizing dose is a large one (10 c.c. of egg-white) the animal, after a short interval (10 to 20 days) is not so thoroughly sensitized as it is when the sensitizing dose is smaller (1 c.c.). For corroboration of this compare Nos. 9, 13, and 21 in Table 3 with Nos. 235, and 355 in the same table; but when the time interval runs up into months, the larger sensitizing dose is quite as effective as the smaller one. However, there are so many factors that must be taken into consideration, and inas-

much as one of these, the individuality of the animal, cannot be measured, we must be careful about drawing conclusions; but we have no doubt that a wider experimental observation will give us a correct explanation. At present we are justified in tentatively suggesting the explanation that it takes the animal a longer time to dispose of a sensitizing dose of 10 c.c. than one of only one-tenth this amount. The explanation of the observed fact, that in animals to which multiple injections have been given the time interval between treatments is of marked importance, will be evident to all who have followed us thus far.

We wish to call attention to the fact that in the development of either sensitization or immunity each proteid apparently has its own individuality and characteristics. The haptophor of the proteid of the colon or typhoid bacillus, provided the dose be a small one, will sensitize a guinea-pig within 30 minutes, so that it will successfully dispose of from four to six times the minimum lethal dose of a living culture of the bacterium; but this sensitization begins to diminish in from three to seven days, and is wholly lost in between 30 and 40 days; while the haptophor of egg-white requires from 10 to 12 days to develop a recognizable degree of sensitization. But the condition having been established, it continues, without apparent diminution for months, possibly for years, and may be transmitted to the offspring of the sensitized animal. We infer from this that the haptophor of the bacterial proteid causes only a slight and temporary modification in the animal cells that supply its specific proteolytic enzymes, while the haptophor of the egg-white produces a profound and lasting effect upon the same animal cells. This is also shown by the fact that the bacterial proteid kills at the first dose, while the egg-white must be given a second time. Each proteid will need to be investigated individually before we can confidently make any statement concerning the amount of it necessary to sensitize, the time that must elapse before the condition of sensitization is established, and the continuance of the condition, and we wish to state that while our method of splitting up proteids in the retort has, in the case of all proteids with which we have worked, yielded a toxophor body, it has with several proteids failed to give a sensitizing or immunizing haptophor. The pneumococcus when broken up with alcoholic alkali gives a poison,

but the non-poisonous portion gives no immunity to the living organism, and so far we have not been able to obtain a sensitizing or immunizing haptophor from casein. This is not at all strange, because casein, as is well known, is quite a different proteid structurally from egg-white. It is probable that with other reagents, or with the same reagents in different proportions, we may get from the cellular substance of the pneumococcus and from casein immunizing and sensitizing proteids, but we wish to state clearly that each proteid will need to be investigated before anything can be predicted concerning the haptophor, and it is possible that there may be proteids that contain no true haptophor groups. We make no claim of the discovery of a universal law.

In conclusion we wish to state briefly the deductions which we have drawn from our work as outlined in this and previous papers.

1. All the proteids with which we have worked may be split up into poisonous and non-poisonous portions.

2. The poisonous group is an essential constituent of all the proteid molecules; and while it is not identical, so far as its chemical structure is concerned, in any two proteids, it is similar in its psysiological action in all the proteids which we have investigated.

3. The poisonous group is the chemical nucleus, i. e., the center of chemism or chemical energy, in the proteid molecule.[21]

4. This group must for the present be regarded as a proteid body, the chemical structure of which remains unknown.

5. It owes its poisonous action to the avidity with which it combines with certain groups in the molecules that constitute the cells of the respiratory center; in other words, it is a respiratory poison.

6. The exact effect of the poisonous group upon an animal depends upon the manner and the rate of its introduction into the body. It may induce acute, subacute, or chronic poisoning.

7. Life is molecular, or metabolism, the essential phenomenon of life, is intramolecular. A bacterium consists essentially of a living proteid, made up of labile molecules that are constantly reacting with outside matter, thus growing and multiplying. The pathogenicity of a bacterium depends upon its ability to multiply in the animal body and convert certain constituents of the animal body into a foreign proteid, and when the foreign proteid thus formed is broken

up in the body of the sensitized animal, its poisonous group is set free and induces the symptoms of disease and death.

8. Proteid susceptibility and immunity are different manifestations of one and the same process. Both depend upon the development in the animal body of a specific proteolytic ferment. When this specific ferment splits up a living foreign proteid before it has time to multiply we say that the animal is immune. When this cleavage action is less prompt, but sufficiently so to split up the living proteid before it elaborates a fatal amount of the poison, the animal sickens, but recovers. When the action of the ferment is still less prompt and the living proteid constructs enough poison to kill, then its liberation causes death. When this specific proteolytic ferment has been developed in the animal by previous treatment with a dead or stable proteid, it is easy to inject a small dose of the same proteid in sufficient quantity to quickly induce symptoms and to kill, then we say that the animal has been sensitized or is in a condition of hypersensibility. With a dead, stable proteid it is easy so to adjust the dose that the animal will show no symptoms, or manifest the first and second stages and recover, or die. With a live or labile proteid the conditions are much more complicated and consequently the result is more uncertain and less controllable.

9. Life is rythmic, i. e., the living, labile molecule reacts with outside matter rythmically, but the *tempo* of this reaction is subject to change when conditions are altered. One strain of the pneumococcus may kill a guinea-pig in a dose of one-millionth of a c.c., while 1 c.c. of another strain may be required to produce the same result. This difference in virulence is, we believe, due to difference in the *tempo* of the molecular reactions, and this introduces into certain cases of infection a variable which at present we have no means of measuring or controlling.[20]

10. In some, not in all, of the proteids with which we have worked the non-poisonous portion has proved to be an immunizing or sensitizing haptophor, giving a specific immunity to its own living, labile proteid, or developing a specific sensitization to its own dead, stabile proteid.

11. These specific haptophors do not sensitize animals to themselves because they contain no poisonous group.

12. Sensitization consists in developing in the animal a specific proteolytic ferment which acts upon the proteid that brings it into existence, and on no other. It may be interesting to state here that we have demonstrated that guinea-pigs sensitized with woman's milk respond to a second treatment with woman's milk and not to cow's milk, and those sensitized to cow's milk do not react with woman's milk.

13. This specific proteolytic ferment stored up in the cells of the animal as a result of the first treatment with the proteid remains in the cells as a zymogen until activated by the second injection of the same proteid.

14. Our conception of the development of a specific zymogen supposes a rearrangement of the atomic groups of the proteid molecules of certain cells in the animal body, or an alteration of the molecular structure. In other words, we regard the production of the specific zymogen not as the formation of a new body, but as resulting from an alteration in the atomic arrangement within the proteid molecule and a consequent change in its chemism.

15. Some proteids, in developing the specific zymogen, produce profound and lasting changes in molecular structure, while the alterations induced by others are slighter and of temporary duration, the molecular structure soon returning to its original condition.

16. In order to serve as a good sensitizer the proteid must be in solution. This is the reason why the haptophor of the colon bacillus sensitizes an animal so much more readily than either the live or the dead bacillus. In fact, as we have seen, the haptophor may sensitize the animal within 30 minutes, so that it will resist from four to six times the minimum or the usual lethal dose of the living bacillus.

17. In treating infected animals the sensitizing haptophor must be employed in minimum doses, only enough to activate the zymogen, because larger quantities consume the activated ferment themselves and thus protect the infecting organism. Ten mg. of the haptophor of the colon bacillus will protect a pig against quantities of the living bacillus (administered 30 minutes later), against which 50 mg. would fail to afford any protection. By minimum doses we do not mean infinitesimally small, and just what they are can be learned only by experiment and experience.

18. What cells are concerned in the elaboration of the specific zymogens we can only surmise at present, but on *a priori* grounds we suppose that they belong to the mesodermal tissue, one of the functions of which, even in the unicellular organism, is to dispose of and utilize proteids of diverse nature.

19. It is possible that studies along this line may be of service in investigating problems of heredity, and substantiate Loeb's statement that heredity is a chemical question.

20. The transmission of the conditions of sensitization seems to be in accord with the view that mutations are not the result of slowly established alterations, but appear suddenly.

BIBLIOGRAPHY.

The following are the more important papers from the Hygienic Laboratory of the University of Michigan bearing on the subject discussed in the preceding pages—

1. VAUGHAN, V. C. and PERKINS, G. D. Ein in Eiscreme und Käse gefundener giftproducirender Bacillus. *Archiv. f. Hyg.*, 1896, 26, p. 308.
2. VAUGHAN, V. C. AND MCCLYMONDS. Bacteriologic Poisons in Milk. *Jacobi Festschrift*, New York, 1900, p. 108.
3. VAUGHAN, V. C. The Bacterial Toxins. *Trans. Assn. Amer. Phys.*, 1901, 16, p. 217.
4. VAUGHAN, V. C. The Toxin of the Colon Bacillus. *Jour. Amer. Med. Assoc.*, 1901, 36, p. 479; *Amer. Med.*, 1901, 1, p. 302.
5. DETWILER, A. J. The Toxicity of the Dry Sterile Cells of Certain Non-Pathogenic Bacteria. *Trans. Assn. Amer. Phys.*, 1902, 17, 246.
6. WHEELER, MAY. The Chemistry of Sarcina Lutea. *Ibid.*, p. 265.
7. LEACH, MARY F. The Chemistry of Bacillus *Coli communis*. *Ibid.*, p. 274.
8. MARSHALL, C. E. AND GELSTON, L. M. The Toxicity of the Cellular Substance of the Colon Bacillus. *Ibid.*, p. 298.
9. GELSTON, L. M. The Intracellular Toxin of the Diphtheria Bacillus. *Ibid.*, p. 308.
10. VAUGHAN, J. W. The Anthrax Toxin. *Ibid.*, 313.
11. WHEELER, MAY. The Action of Mineral Acid on the Cellular Substance of *Bacillus typhosus*. *Jour. Amer. Med. Assoc.*, 1904, 42, 1000.
12. LEACH, MARY F. The Chemistry of Bacillus *Coli communis*. *Ibid.*, p. 1003.
13. MCINTYRE, D. The Intracellular Toxin of *Bacillus pyocyaneus*. *Ibid.*, p. 1073.
14. MUNSON, J. F. AND SPENCER, F. R. The Extraction of a Toxin from Liver Cells. *Ibid.*, p. 1075.
15. VAUGHAN, V. C. Further Studies on the Intracellular Bacterial Toxins. *Jour. Amer. Med. Assoc.*, 1905, 43, p. 643.
16. WHEELER, MAY. The Extraction of the Intracellular Toxin of the Colon Bacillus. *Ibid.*, 44, p. 1271.
17. VAUGHAN, V. C., JR. The Action of the Intracellular Poison of the Colon Bacillus. *Ibid.*, p. 1340.

18. VAUGHAN, V. C., JR. The Production of Active Immunity with the Split Products of the Colon Bacillus. *Jour. Med. Res.*, 1905, 14, p. 67.

19. LEACH, MARY F. On the Chemistry of Bacillus *Coli communis*. *Jour. Biol. Chem.*, 1906, 1, p. 463.

20. VAUGHAN, V. C. Some New Conceptions of the Living Cell; Its Chemical Structure and Its Functions. *Canadian Jour. Med. and Surg.*, 1905, 18, p. 283.

21. VAUGHAN, V. C. A Contribution to the Chemistry of the Bacterial Cell and a Study of the Effects of Some of the Split Products on Animals. The Shattuck Lecture, 1906. *Boston Med. and Surg. Jour.*, 1906, 155, p. 215.